DNA methylation-based classification and grading system for meningioma: a multicentre, retrospective analysis

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Molecular analysis of meningioma increases prognostic power: A methylation-based classification and grading system

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Summary

Background

The World Health Organization (WHO) classification of brain tumors describes 15 subtypes of meningioma. Nine of these are allotted to WHO grade I, and three each to grade II and grade III, respectively. Grading is purely based on histology, with molecular markers lacking. While the current classification and grading approach is of prognostic value, it harbors shortcomings such as ill-defined parameters for subtypes and grading criteria prone to arbitrary judgment.

Methods

We investigated genome-wide DNA methylation patterns of 479 meningiomas to identify distinct methylation classes (MC) of meningioma. The MCs were further characterized by DNA copy-number analysis, mutational profiling and RNA sequencing. We validated our findings in an independent cohort of 140 tumors.

Findings

DNA methylation profiling distinguished six distinct MCs associated with typical mutational, cytogenetic, and gene expression patterns. Meningioma MCs exhibit a more homogeneous clinical course and allow prognostication with significantly higher power than the current morphology-based WHO classification. Meningioma MCs more accurately identify patients at high risk of recurrence among tumors with WHO grade I histology, and patients at lower risk of recurrence among WHO grade II tumors. DNA methylation-based classification and grading reduces the number of meningioma subtypes from 15, as historically defined by histology, to six clinically relevant MCs, each with a characteristic molecular and/or clinical profile.

Interpretation

DNA methylation-based meningioma classification captures biologically more homogenous groups and has a higher power for predicting tumor recurrence than the current WHO classification. The approach presented here is highly useful for stratifying meningioma patients for observation or adjuvant treatment groups. We consider methylation-based tumor classification highly relevant for the future diagnosis and treatment of meningioma.

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Research in context

Evidence before this study

Meningiomas, the most frequent primary intracranial tumors, are diagnosed and graded according to the WHO classification of brain tumors. The recent update of this classification in 2016 has implemented molecular markers for several brain tumor entities.

However, there are still no established prognostic molecular markers for meningioma. Meningioma diagnostics is still based on purely histological criteria which are prone to a high inter-observer and sampling bias. Thus, the relevance of the current grading system for clinical decision making is heavily debated.

Previous work by several groups, including ours, has shown that DNA methylation signatures are specific for tumor entities. Importantly, DNA methylation profiling can identify biologically and clinically relevant subgroups among histologically indiscernible cases. Here, we employed this concept for the classification of meningiomas. A search in PubMed on October 21 2016 did not identify articles which used high-resolution DNA methylation profiling for identification of clinically relevant subgroups across all subtypes and grades of meningioma.

Added value of this study

We demonstrate that classification of meningiomas based on DNA methylation profiling is more powerful in predicting the clinical behavior than the current WHO classification and grading system. Our findings on a discovery series were confirmed on an independent validation series. Most notably, the novel approach was capable of identifying patients at high risk of rapid recurrence which were expected to have benign tumors based on WHO grading. Likewise, a considerable fraction of patients with the histological diagnosis of a higher grade meningioma - fostering the consideration of adjuvant treatment - but no recurrence could upfront be identified as low risk by DNA methylation profiling.

Implications of all the available evidence

Our data demonstrate that meningioma patients can be more accurately stratified for tumor behavior by DNA methylation profiling than by the current WHO classification. This greatly improves the basis for clinical decision making for or against additional therapy after surgery. We expect epigenetic profiling to be included into the diagnostic routine and implemented into upcoming updates of the WHO classification for brain tumors.

Introduction

The meninges exert a protective function for the entire central nervous system (CNS). During development, their precursor cells emerge from mesodermal structures and the neural crest, actively contributing to the differentiation of the brain[1-3]. However, meningeal cells may transform to initiate tumors. These meningiomas are the most frequent primary intra-cranial and spinal tumors [4]. While 80 % of meningiomas show a benign clinical behavior and can be cured by resection alone, about 20 % recur and need additional treatment such as repeated surgery, irradiation, and systemic chemotherapy[4,5]. Histopathological evaluation aims at the identification of cases at risk for recurrence. The histological differentiation into subtypes initially dates back to the 19th century. Later, in a first internationally recognized classification approach in 1928, Bailey and Cushing distinguished meningothelial, fibroblastic, and angiomatous subtypes [6], and to this day, allocation to subtype is based solely on histological findings. The current WHO classification recognizes 15 subtypes and three grades of malignancy[4], but some of the diagnostic criteria are vaguely defined and subject to a high inter-observer bias, indicating the need for more reliable markers[5,7].

For various other CNS tumors, molecular profiling has identified distinct subtypes with characteristic aberrations. Many of these correlate with prognosis or provide targets for treatment, and therefore support clinical decision making, e.g. epigenetic subgroups in medulloblastoma[8-10] and ependymoma[11], or *isocitrate dehydrogenase* (*IDH*) status in diffuse glioma[12-14]. Recent studies identified *telomerase reverse transcriptase* (*TERT*) promoter mutations in a small subset of meningiomas to be associated with higher risk of recurrence and shorter time to progression[15,16], and four large exome-sequencing efforts focusing on WHO grade I meningiomas have identified recurrently mutated genes beyond the long-known association with *NF2* [17-20]. Yet, these findings cover only a fraction of meningiomas and have not all been thoroughly tested for their prognostic relevance. In this study, we aimed at a comprehensive characterization of the entire molecular genetic landscape of meningioma in order to identify biologically and clinically relevant subgroups that refine the current classification scheme.

Results

DNA methylation analysis identifies six distinct methylation classes of meningioma

We generated genome-wide DNA methylation profiles from a discovery cohort of 497 meningiomas (Suppl. Fig. 1) along with 309 samples of other extra-axial skull tumors that may histologically mimic meningioma variants, including solitary fibrous tumor/hemangiopericytoma, schwannoma, malignant peripheral nerve sheath tumors, chordoma, chondrosarcoma, fibrous dysplasia, and hemangioblastoma. Despite sharing a mesodermal origin, unsupervised clustering of DNA methylation data clearly segregated all meningiomas from other skull tumors (Suppl. Fig 2). Unsupervised clustering of meningiomas alone revealed two major epigenetic groups (Groups A and B, Fig. 1A), with both groups further subdividing into four and two subgroups, respectively. These six subgroups were designated as "methylation classes" (MCs). Based on further molecular and clinical characteristics outlined below, the four MCs of Group A were designated MC benign 1 through 3 (MC ben-1, ben-2, ben-3) and MC intermediate A (MC int-A). The two MCs of Group B were designated MC intermediate B (MC int-B), and malignant (MC mal).

There was an enrichment of grade I tumors among MC ben-1, MC ben-2, and MC ben-3, and an enrichment of WHO grade III tumors in MC mal, while WHO grade II tumors where scattered across all MCs. Analysis of 75 primary and matched recurrent tumors from 37 patients showed that association with Group A or B was stable upon recurrence (Fig. 1B), supporting further assessment of methylation profiling for diagnostic and prognostic implications.

MC predict clinical course with higher accuracy than WHO grading

The wide spectrum of clinical behavior among WHO grade I and II meningiomas points towards the limited prognostic power of the current classification, particularly at the border between grade I and II. As a result, the basing of decisions about radiotherapy on the current grading scheme is heavily debated [5]. Thus, we correlated meningioma MCs with progression-free survival (PFS) to evaluate their potential for predicting outcome compared to WHO grading (Fig. 2A, B). We further combined MCs exhibiting virtually identical benign (MC ben-1, MC ben-2, MC ben-3) or intermediate (MC int-A, MC int-B) outcome into combined MCs (Fig. 2C). Classification by individual and combined MCs demonstrates more precise prognostication than by WHO grading (Fig. 2D, Brier prediction test, $p < 0{\cdot}01$). These findings were confirmed in 140 meningiomas from an independent validation cohort (Suppl. Fig 3A, B).

We next focused on the prediction power of MCs within WHO grades and, particularly, patients divergently diagnosed by WHO grading and DNA methylation-based classification. Patients with WHO grade I meningiomas who were molecularly assigned to an intermediate MC experienced a less

favorable clinical course than patients with WHO grade I meningiomas diagnosed solely based on histology. In fact, their outcome was indistinguishable from that of patients with WHO grade II meningiomas (Fig. 3A). Likewise, patients with WHO grade II meningiomas molecularly assigned to a benign MC had a better outcome than the average outcome of patients with histologically-defined WHO grade II meningiomas. Consequently, stratification for MC is of higher value for prediction of PFS than WHO grading. Within the combined MCs, WHO grading confers limited additional information (Fig. 3B, Suppl. Table 2). However, combined MCs delineate subgroups with significantly distinct prognosis within all WHO grades (Fig. 3C), demonstrating the benefit of MC-based grading for patients and the potential to significantly reduce under- or overtreatment.

Methylation classes are associated with distinct driver mutations and copy-number-alterations

We next sequenced 304 meningiomas with sufficient material available using a custom hybrid-capture next-generation sequencing (NGS) panel dedicated to 40 genes previously reported to be mutant in meningioma (Suppl. Table 1), based on our recently established custom NGS approach for routine brain tumor diagnostics[21]. Known recurrent mutations (most frequently *NF2*, followed by *TRAF7* and *AKT1*) were significantly enriched in certain MCs (Suppl. Table 3, Fig. 4). Within Group A, *NF2* mutations were observed in 63 % of MC ben-1 tumors (significant accumulation of parameter in this MC, p <0·0001, Fisher's exact test). MC ben-2 contained the vast majority of meningiomas carrying *AKT1* (33 % in this subgroup; p < 0·0001), *SMO* (7 %; p =0·0002), *KLF4* (15 %; p < 0·0001), and *TRAF7* (49 %; p < 0·0001) mutations, but only rarely harbored *NF2* mutations. Only one *AKT1* and five *KLF4* mutations were detected outside of MC ben-2. MC ben-3 exhibited *NF2* mutations in 32 % and *PIK3CA* mutations in 11 % of tumors, representing the majority (5/7, 71 %) of *PIK3CA* mutations in the cohort. MC int-A carried *NF2* mutations in 53 %. Within Group B, MC int-B tumors harbored *NF2* mutations in 35 % and MC mal in 31 % of cases. *SUFU* mutations were confined to Group B, with 5 % of MC int-B and 6 % MC mal tumors being mutated. Four out of five *TERT* promoter mutations mapped to meningiomas in Group B (p = 0·005, Fisher's exact test).

Annotation of copy-number-variations (CNV) revealed that MCs are associated with distinct cytogenetic aberrations (Fig. 4A, B): MC ben-1 was associated with deletions of 22q (95 %) but otherwise virtually no CNV. MC ben-2 presented with absence of recurrent CNVs. Typical for MC ben-3 were multiple chromosomal gains, most frequently affecting chromosome 5 (47 %). MC int-A frequently exhibited losses on 1p (70 %) and 22q (84 %). In Group B, MC int-B frequently exhibited losses on 1p (89 %), 10 and 22 (89 %), all features also shared with MC malignant. However, in MC mal, a higher frequency of *CDKN2A* deletion was apparent (70%).

Representative cases with sufficient material available of all MCs underwent RNA-sequencing which identified differentially upregulated genes and pathways (Suppl. Fig. 4).

Methylation classes and WHO subtypes, localization, and gender

Examining the distribution of histological subtypes, which currently determine grading, across MCs revealed which histological subtypes the MCs are composed of and, conversely, to which MC the samples of a respective subtype are assigned (Fig. 5). The rare lymphoplasmacyte-rich meningioma (WHO grade I) was not assessed due to the overwhelming dominance of constitutional (non-tumor) DNA in these samples. In general, two patterns were observed: Either a given MC was strongly associated with a small set of or even single histological subtypes, or samples of a particular MC or subtype were widely spread across all corresponding variants. MC ben-1 comprised the majority of fibroblastic meningiomas and is also enriched for psammomatous meningioma. Fibroblastic meningiomas frequently harbor calcifications called psammoma bodies, and a high abundance of these calcifications defines psammomatous meningioma. Their molecular similarity implies that they may rather represent a continuous spectrum of one phenotypic pattern. The overexpression of SERPINF1 (Suppl. Fig. 4), which has been implicated in osteogenesis and calcification, in MC ben-1 might contribute to this histologically detected phenomenon. MC ben-2 was highly enriched for meningothelial meningiomas, and contained the vast majority of secretory meningioma. MC ben-3 harbored cases from several subtypes but was particularly enriched for angiomatous meningiomas. This is in line with the overexpression of vessel-associated markers, e.g. factor VIII, in samples of this MC (Suppl. Fig. 4). Transitional meningioma, a hybrid of meningothelial and fibroblastic histology, dissolved into several MCs, along with the samples of the rare microcystic, chordoid, metaplastic, psammomatous, and clear cell subtype.

The two intermediate MCs were predominantly constituted of atypical meningiomas. However, a considerable fraction of atypical meningioma (n=31) fell into MC ben-1. The improved resolution of this group substantially contributes to the higher prognostic power of MC class over histology. Anaplastic meningiomas predominantly mapped to MC malignant. Of note, the six rhabdoid/papillary meningiomas, by definition WHO grade III, all ended up in one of the MC benign or intermediate meningioma groups. However, the number was too low to assess the statistical relevance of WHO grading and MC classification individually for rhabdoid/papillary meningioma.

Transitional meningioma WHO grade I was much more frequently assigned to an intermediate MC than fibroblastic or meningothelial meningioma, mostly to MC int-A. Atypical meningiomas assigned to a benign MC accumulated in MC ben-1.

The most frequent localizations for all subgroups were the frontal and central convexity, except for MC ben-2 (Fig 6). For the latter, basal localization was common, in line with the high occurrence of *AKT1* and *SMO* mutations in this MC which are known to be enriched in this location [19,22]. Interestingly, all MC mal cases were located at the convexity. In contrast, none of the basal tumors

were allotted to MC mal, including four intraventricular and ten spinal meningiomas that were all assigned to intermediate or benign MCs. Age distribution was equal throughout all MCs. In terms of gender, we observed a predominance of male patients in MC mal, while all other MCs mainly comprised female patients (Fig 6).

Discussion

The 15 subtypes of meningioma included in the current WHO classification have evolved over decades. The major aim of introducing this variety of subgroups was to cover the whole histological spectrum of meningioma and to avoid misclassification of tumors mimicking other entities. For example, meningeal tumors with chordoid or rhabdoid cytology may initially raise suspicion of a chordoma or rhabdoid tumor but not point towards meningioma. Therefore, particular subtypes with these features were introduced into the classification in order to draw attention to the morphologic diversity of meningioma. In addition, some cytological features have been reported to be associated with distinct outcome. Although this was based on small series, it prompted allotment of distinct WHO grades to specific meningioma subtypes. However, this approach has been increasingly questioned due to suboptimal inter-observer reproducibility and limited prognostic effect of the histological criteria of higher grade [7,23,24], most recently in a large Radiation Therapy Oncology Group (RTOG) meningioma trial [7] in which the authors expressed the urgent need for more objective, molecular stratification markers.

This resulted in an overall critical view of clinicians with respect to the current meningioma WHO classification and grading, which has been expressed in the most recent published European Association of Neuro-oncology (EANO) guideline for the diagnosis and treatment of meningiomas[5]. Accordingly, revisiting meningioma diagnostics based on DNA methylation profiling by defining MCs with enhanced predictive power will greatly improve the acceptance of meningioma classification and more successfully guide decisions regarding postoperative treatment. An overview on the molecular and clinical hallmarks of the six meningioma MCs is given in Figure 6.

Distinct methylation profiles suggest different development

Beyond the identification of clinically relevant groups and the basis for a novel classification, our dataset might provide insight into the development of meningioma. This has previously been shown for other entities: Four variants of medulloblastoma, distinguishable by their DNA methylation patterns, were shown to arise from different precursor cell populations[8,25-27], and exhibit very different clinical characteristics and therapy needs. Our data indicate that the spectrum of meningiomas is divided into two major molecularly highly distinct Groups (A and B, Fig. 1). This strong separation suggests either the existence of distinct cells of origin or an underlying event with major impact on genome-wide DNA methylation. The very different DNA methylation profiles of Groups A and B despite the shared occurrence of *NF2* mutations might suggest that meningiomas arise from two different precursor cell populations. Based on our own and published high-throughput sequencing data, there is no evidence for the existence of a single mutation being solely responsible for the separation of these two groups. However, we cannot fully exclude the existence

of alterations not readily detectable by these approaches, such as translocations or fusions, causing the responsible changes in the methylome. Moreover, the fact that patients with meningiomas clustering in Group A share a predominantly benign, with a small proportion exhibiting a intermediate clinical course, and that patients with meningiomas of Group B follow an intermediate to malignant clinical course, may further argue towards a distinct cell of origin with different intrinsic propensities for malignant transformation. However, analyses dissecting the full regulatory background of the tumor cells in comparison to arachnoidal cells, e.g. by H3K27Ac ChIP-Sequencing, are needed to further elucidate this.

Methylation-based versus WHO subgrouping versus other molecular markers

Extensive whole exome or -genome sequencing has provided a large body of information on the mutational landscape of meningioma[17-20]. Four distinct meningioma mutational subgroups have been proposed, defined by mutations either in *NF2*, *TRAF7*, the hedgehog pathway, or *POLR2A*[18]. However, such a model of meningioma development based on mutational analysis alone currently does not satisfy the clinical need for distinction between patients in need of adjuvant treatment or not. A major drawback is the lack of risk stratification among *NF2*-mutant cases, which can present with any clinical course. While the strong association of *AKT1*, *TRAF7*/*KLF4*, or *SMO* mutations with benign, or *TERT* promoter mutations with unfavorable course may allow for mutation-based risk assessment in these subgroups, the current inability to stratify *NF2*-mutated meningiomas for other mutational events associated with clinical outcome is a major obstacle for a classification and grading system based on mutational profiling alone.

Similarly strong limitations apply to approaches based on copy-number-profiles: They leverage the accumulation of aberrations during progression but are not capable of predicting the behavior upfront. The current dataset attributes the highest prognostic power to methylome-based subgrouping, which proves to be superior to WHO classification (Fig. 2, 3), while an exclusively mutation-based subgrouping for the full spectrum of meningioma is not available.

An integrated diagnosis for meningioma evaluation

The WHO 2016 revision of the classification for CNS tumors supports the concept of an integrated diagnosis. It relies on a multilayered approach combining data from histology, molecular genetic analyses, and clinical findings[4,28,29]. Adopting this WHO approach to the diagnosis of meningioma, the morphological layer corresponds to the current diagnostic standard, i.e. diagnosing the 15 WHO meningioma subtypes and grading according to the morphological scheme. In the absence of molecular analyses the morphological diagnosis should be suffixed with NOS (not otherwise

specified), as agreed for parenchymal brain tumors without molecular workup[4]. The molecular diagnostic layer may contain elements such as DNA methylation and/or mutation analyses. Mutational data may enable inferring the MC for a subset within the MC ben-2, e.g. for *AKT1* mutant cases, but not in every instance. With methylation analysis performed, one of the six MCs can be diagnosed. If the MC is identified, this results in a significantly more powerful prediction of the clinical course. This corresponds to the current approach in other entities, e.g. ependymoma and medulloblastoma, for which methylation profiling has proven to be more relevant than histological grading[4,11]. Based on the data presented here, the integrated diagnosis of meningioma will also highlight the prognostic impact of MCs, but in addition refer to the morphological subtype identified in histological examination.

Collectively, the dataset and accompanying classification scheme proposed here advances meningioma diagnostics from histology into an integrated profiling with higher accuracy of risk assessment for individual patients.

Author contributions

FS and AvD conceived the project, coordinated data generation, and wrote the manuscript with input from all co-authors. FS, D Schrimpf, D Stichel, DTWJ, SS, D Sturm, M Sill, VH, LC and SMP designed methylation experiments and analyzed DNA methylation data. FS, D Schrimpf, D Stichel, LC and KO analyzed RNA sequencing data. FS, D Schrimpf and TH analyzed survival data. FS, D Schrimpf, D Stichel, SS, DR, CK, DC, KH, AK, AW analyzed DNA sequencing data. M Schick and MBH performed array experiments. FS, DR, CK, DC, KH, AK, AW, PB, K Kurian, AFO, CM, AK, JS, EJR, VPC, WP, MM, and AvD performed histological evaluation. HGW, ASB, PB, HE, K Kurian, AFO, CM, CJ, KD, MSR, RK, M Simon, AB, M Westphal, KL, AK, JS, VPC, SB, M Platten, DH, AU, WP, WW, MM, M Preusser, CHM, and M Weller collected and interpreted clinical data and/or compiled respective tissue collections.

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Materials and Methods

Samples

Samples with clinical data were retrospectively collected from the Dept. of Neuropathology Heidelberg, Germany (local and referral cases), Dept. of Neurosurgery Heidelberg and the FORAMEN network, the Dept. of Neurology and Neuropathology, Zürich, Switzerland, and the Neurological Institute (Edinger Institute) Frankfurt/Main, Germany. Additional samples without survival annotation were included from the Dept. of Neuropathology and Neurosurgery Berlin, Bonn, Hamburg, Magdeburg, Münster, Tübingen (all Germany), and Bristol (UK). The validation cohort was provided by the Medical University of Vienna. Sample and data collection was in accordance with local ethical regulations.

Methylation analysis, copy-number analysis

Unsupervised clustering of the 450k data of the discovery and the EPIC 850k data of the validation cohort was performed as previously described (see Supplementary Materials and Methods for references) based on Euclidian distance and Ward's linkage method. For the clustering probes with a standard deviation greater 0.2 across all samples were selected. The methylation probes were reordered by hierarchical clustering using Euclidean distance and complete linkage.
Copy-number aberrations were inferred from methylation array data using the R/Bioconductor package conumee.

Cohort-wide copy number analysis in MCs

Methylation-class wide relative copy-number assessment was performed based on 450k data by a proprietary algorithm and controlled by manual inspection of the conumee-based copy-number-profiles (Stichel et al., in preparation).

Panel and RNA sequencing

Panel sequencing for genes reported to be mutant in meningioma (Suppl. Table 1) was performed applying a custom hybrid-capture approach (Agilent) as described before. RNA libraries were generated with TruSeq RNA Access (Illumina) applying manufacturer supplied protocols. Sequencing was performed on a NextSeq 500 (Illumina).

Statistical analysis of clinical parameters

Distribution of survival times was estimated by the method of Kaplan and Meier and compared between groups with the log-rank test. Hazard ratios including 95% confidence intervals based on Cox regression models were calculated. For the multivariable Cox regression model, imputations of missing covariate values was done applying the multivariate imputations using chained equations

(mice) algorithm with 100 imputation runs. Hazard ratio for age is given per 10 year increment. Prediction error curves based on the Brier score were computed. Integrated Brier score was tested between risk stratifications using 1000 bootstrap samples. P-values below 0.05 were considered statistically significant. Analyses were performed with statistical software R 3.3.

Legends

Figure 1 Unsupervised clustering of methylation data of 479 meningioma samples (A). Unsupervised clustering of matched primary and recurrent samples (matched primary/recurrent samples of identical patient identified by arrows) combined with reference samples from group A and B shows that no shift between groups occurs upon recurrence (B).

Figure 2 Progression free survival (PFS) of 228 case with clinical data stratified for WHO grade (A), methylation class (B), combined methylation classes (C). Brier prediction plot calculated for the models A-C (D, WHO vs combined MCs p=0·0138, 0·0096, 0·0062 for 5, 10 and 12 years, respectively).

Figure 3 Comparison of WHO grading and methylation-based risk prediction: WHO grade I cases allotted to an intermediate methylation class show PFS similar to the average grade II tumors. In turn, WHO grade II cases assigned to a benign methylation class have longer PFS than the average WHO grade II cases (A). Hazard ratio (including 95% confidence intervals) forest plot for WHO grading, overall and stratified for combined methylation classes (B). Hazard ratio forest plot for combined methylation classes, overall and stratified for WHO grading (C). While sub-stratification for WHO grade among MCs is of limited additional value (B), MCs stratify for distinct PFS within WHO grades (C).

Figure 4 Distribution of mutations across sample that underwent panel-sequencing (304) stratified for MCs (A). Copy number variations across all samples that underwent 450k analysis (497) the MCs (B).

Figure 5 Association of histological subtypes and MCs.

Figure 6 Schematic overview over the six identified MCs and their molecular and clinical characteristics

Supplementary Materials (online)

Supplementary Figure 1: Composition of Discovery Cohort (WHO grade, subtype, age, localization, gender)

Supplementary Figure 2: Unsupervised clustering of 450k methylation data of mesenchymal skull tumors and meningioma

Supplementary Figure 3: Kaplan-Meier analysis for Validation cohort stratified for WHO grade (A) and Methylation Class (B)

Supplementary Figure 4: Expression analysis based on RNA-sequencing data. Most differentially expressed genes in the six MCs (A) and ClueGo based on KEGG source data all samples (B). Nodes represent enriched gene sets, which are grouped and annotated by their similarity. Size is proportional to the number of involved genes. Manual curation was performed to remove uninformative sub-networks.

Supplementary Materials and Methods: Detailed methods with references

Supplementary Table 1: Multi-variate analyses

Supplementary Table 2: Genes included in panel sequencing

Supplementary Table 3: Distribution of mutations in Methylation classes

Supplementary Table 4: Clinical and mutational data of all cases

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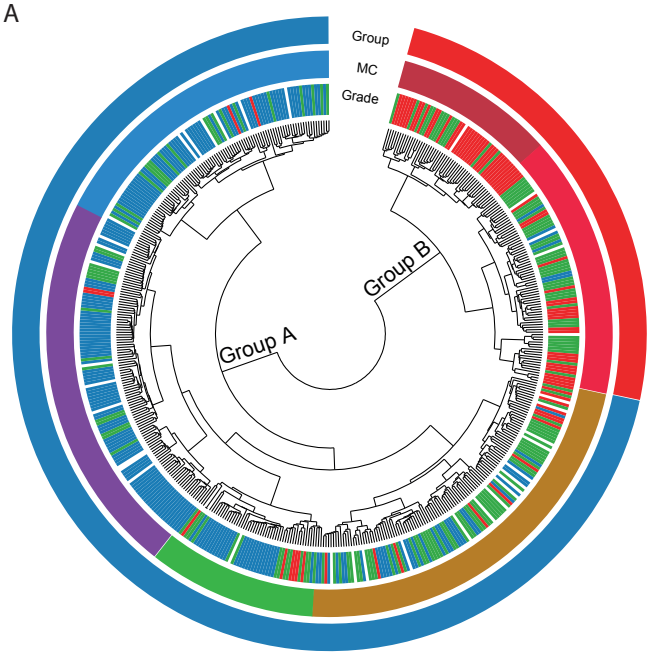
A

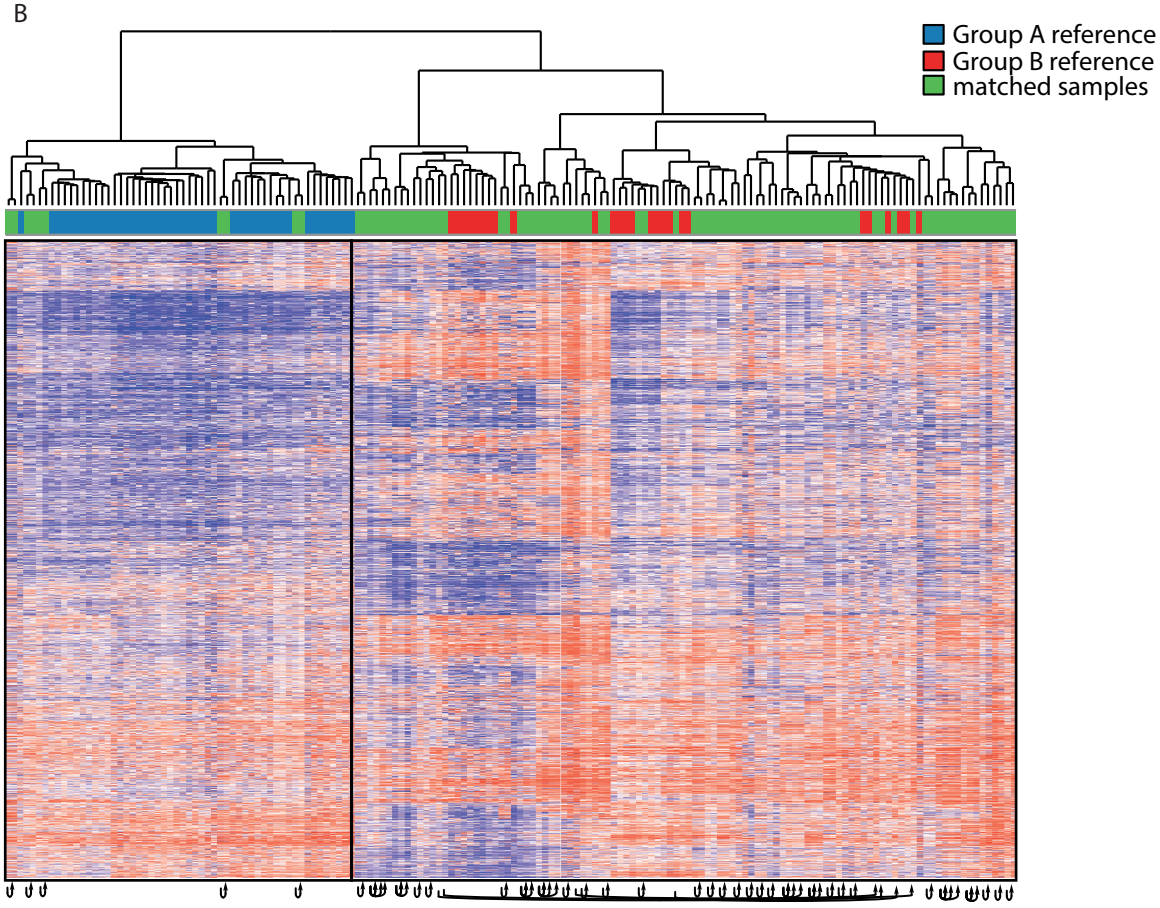


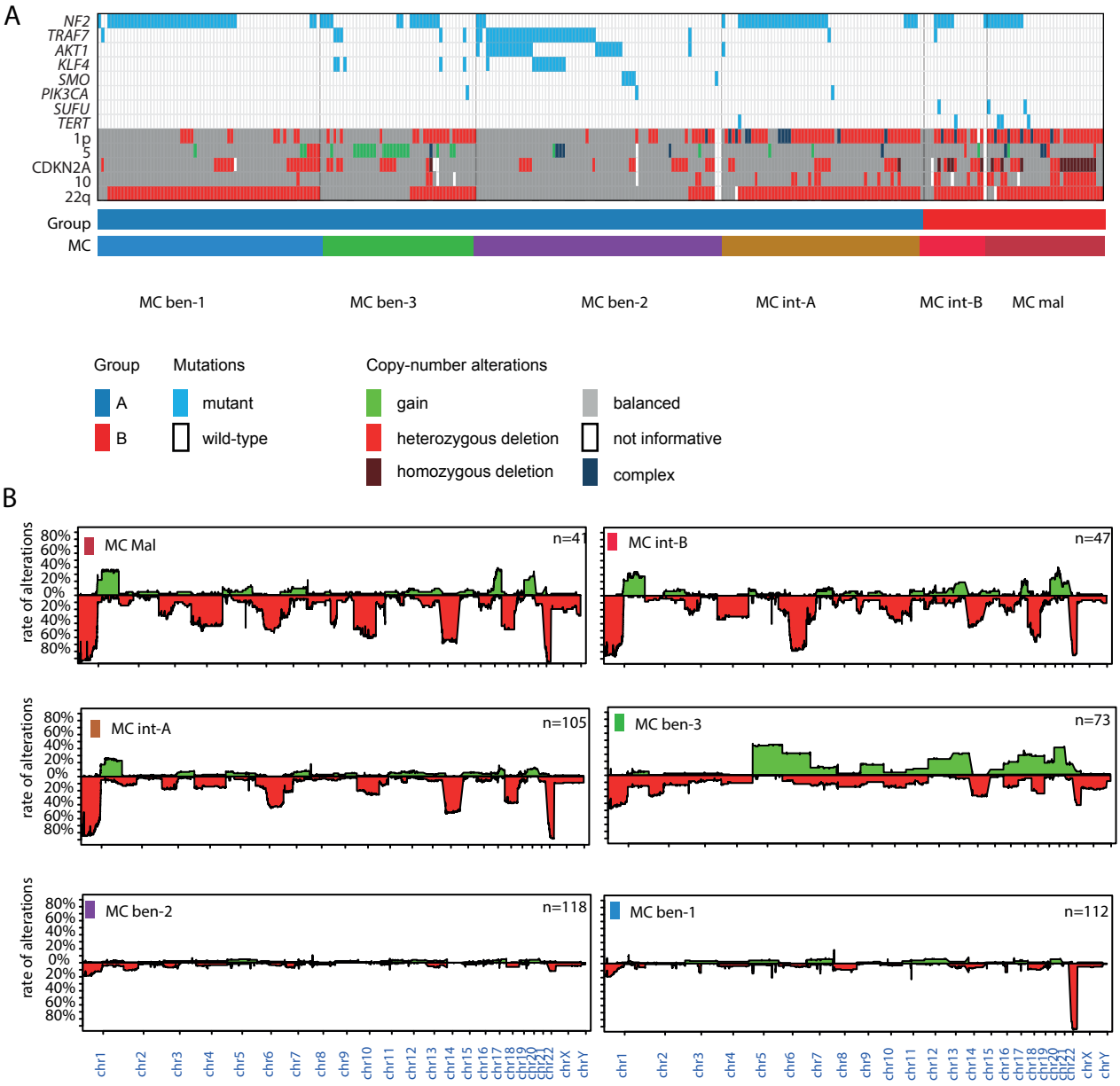
Group
A B

Methylation Class (MC)
MC ben-1 MC ben-2 MC ben-3
MC int-A MC int-B MC mal

Grade
I II III

B




A
NF2
TRAF7
AKT1
KLF4
SMO
PIK3CA
SUFU
TERT
1p
5
CDKN2A
10
22q
Group
MC
MC ben-1
MC ben-3
MC ben-2
MC int-A
MC int-B
MC mal
Group
A
B
Mutations
mutant
wild-type
Copy-number alterations
gain
heterozygous deletion
homozygous deletion
balanced
not informative
complex
B
rate of alterations
MC Mal
n=41
MC int-B
n=47
MC int-A
n=105
MC ben-3
n=73
MC ben-2
n=118
MC ben-1
n=112
chr1
chr2
chr3
chr4
chr5
chr6
chr7
chr8
chr9
chr10
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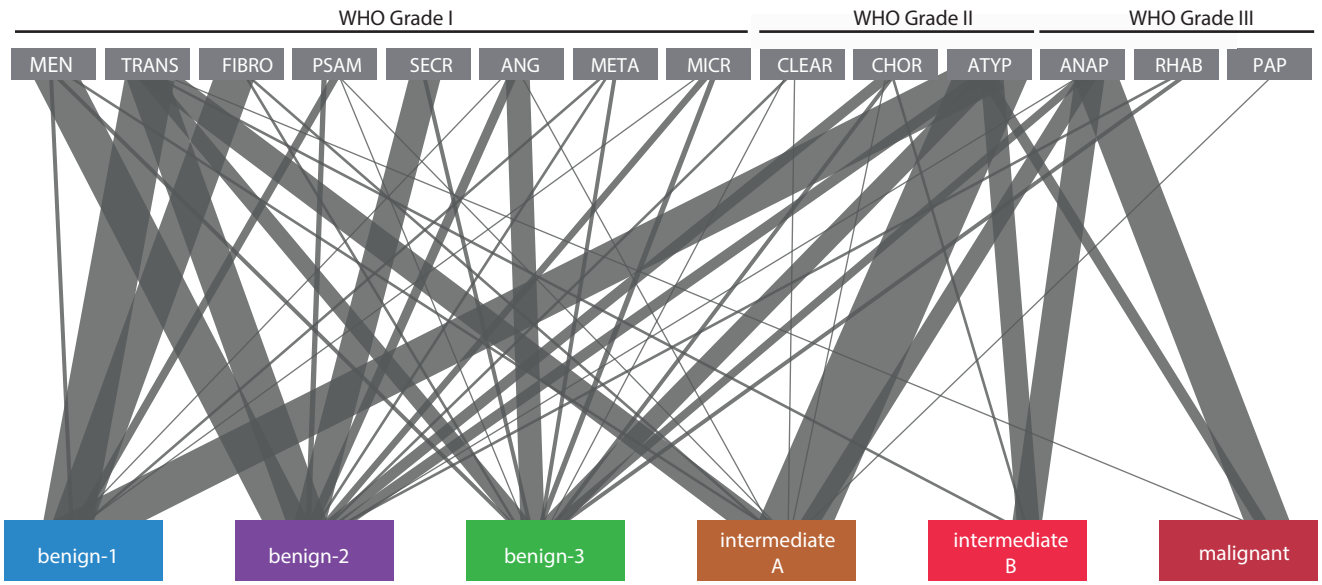
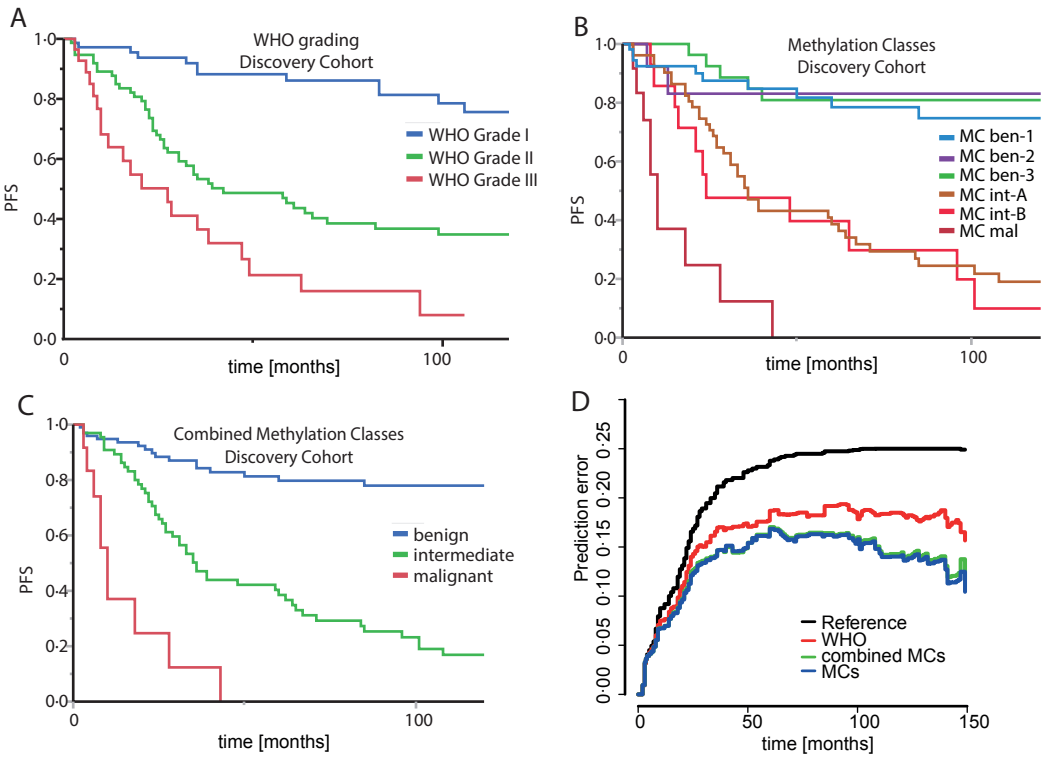
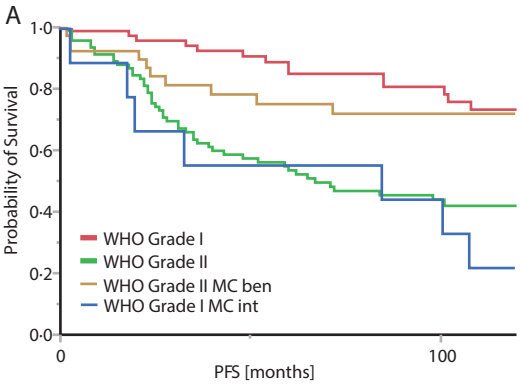
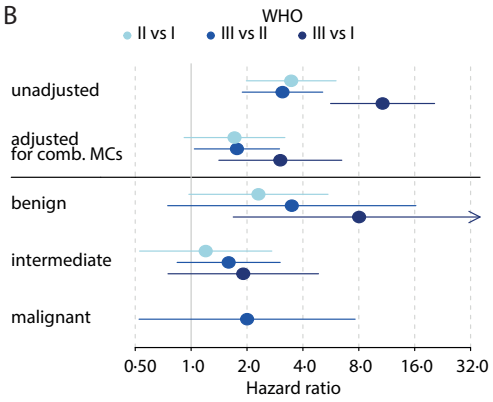
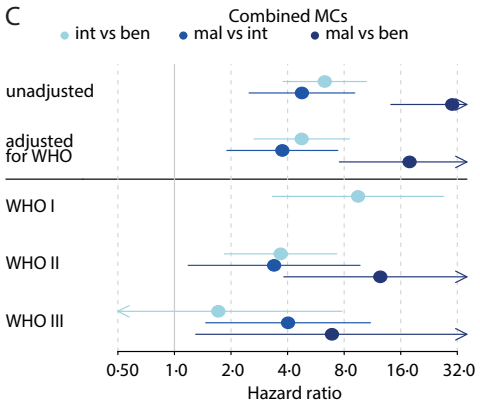
chr21
chr22
chrX
chrY

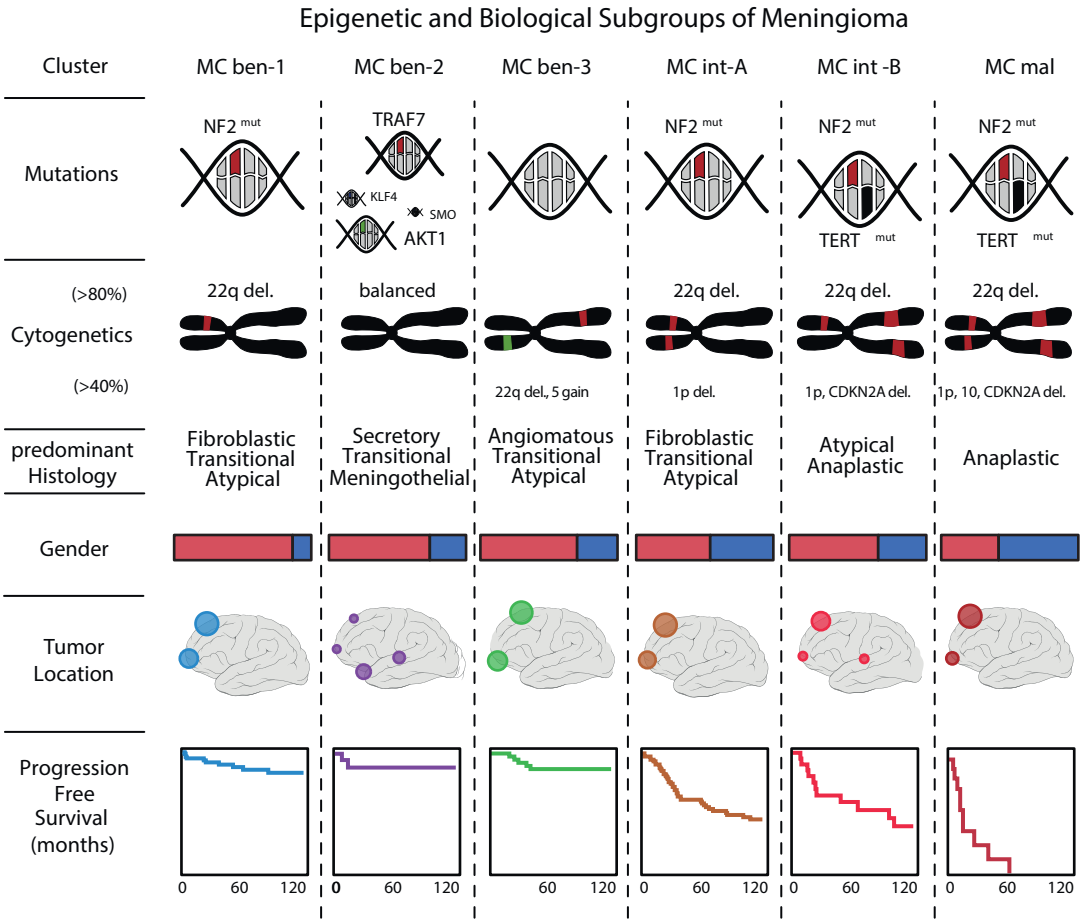

WHO Grade I
WHO Grade II
WHO Grade III
MEN
TRANS
FIBRO
PSAM
SECR
ANG
META
MICR
CLEAR
CHOR
ATYP
ANAP
RHAB
PAP
benign-1
benign-2
benign-3
intermediate A
intermediate B
malignant


A
WHO grading
Discovery Cohort
WHO Grade I
WHO Grade II
WHO Grade III
PFS
time [months]
B
Methylation Classes
Discovery Cohort
MC ben-1
MC ben-2
MC ben-3
MC int-A
MC int-B
MC mal
PFS
time [months]
C
Combined Methylation Classes
Discovery Cohort
benign
intermediate
malignant
PFS
time [months]
D
Prediction error
Reference
WHO
combined MCs
MCs
time [months]


A
1·0
0·8
0·6
0·4
0·2
0·0
Probability of Survival
WHO Grade I
WHO Grade II
WHO Grade II MC ben
WHO Grade I MC int
0
100
PFS [months]


B
WHO
II vs I
III vs II
III vs I
unadjusted
adjusted
for comb. MCs
benign
intermediate
malignant
0·50
1·0
2·0
4·0
8·0
16·0
32·0
Hazard ratio


C
Combined MCs
int vs ben
mal vs int
mal vs ben
unadjusted
adjusted
for WHO
WHO I
WHO II
WHO III
0·50
1·0
2·0
4·0
8·0
16·0
32·0
Hazard ratio


Epigenetic and Biological Subgroups of Meningioma
Cluster
MC ben-1
MC ben-2
MC ben-3
MC int-A
MC int -B
MC mal
Mutations
NF2 mut
TRAF7
KLF4
SMO
AKT1
NF2 mut
NF2 mut
TERT mut
NF2 mut
TERT mut
(>80%)
Cytogenetics
(>40%)
22q del.
balanced
22q del., 5 gain
1p del.
22q del.
1p, CDKN2A del.
22q del.
1p, 10, CDKN2A del.
predominant Histology
Fibroblastic Transitional Atypical
Secretory Transitional Meningothelial
Angiomatous Transitional Atypical
Fibroblastic Transitional Atypical
Atypical Anaplastic
Anaplastic
Gender
Tumor Location
Progression Free Survival (months)
0
60
120